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A. D. Emmett

CHEMISTRY OF FLESH

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FURTHER STUDIES ON THE APPLICATION OF FOLIN'S CREATIN AND CREATININ METHOD TO MEATS AND MEAT EXTRACTS.

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Since the publication of the first paper[2] from this laboratory upon the determination of creatin and creatinin in meats and meat extracts further studies have been made to test the accuracy of the method formerly used. This additional work has been in part prompted by the paper of Otto Hehner,[3] who takes the attitude that Folin's creatin method if directly applied to commercial meat extracts does not give accurate results. Hehner's chief criticism is that 15 cc. of the 1.2 per cent picric acid solution used in Folin's method as applied to the determination of creatin in urine is not sufficient for the determination of this constituent in commercial meat extracts. In his article Hehner states that in such cases an increased quantity of the picric acid solution should be used and he recommends the use of a total of 25 cc. of a 1.01 per cent solution of picric acid together with "a quite small amount of alkali," and he says further that "an excessive quantity of the alkali diminishes the color." This difference in the quantity and strength of the picric acid which is represented by 6.1 cc. of a 1.2 per cent solution, gave results according to Hehner which showed commercial meat extracts (Lemco, Lazenty's, Armour's

[1] H. S. Grindley: *Journ. of the Amer. Chem. Soc.*, xxvi, p. 1086, 1904; H. S. Grindley and A. D. Emmett: *Ibid.*, xxvii, p. 658, 1905; A. D. Emmett and H. S. Grindley: *Ibid.*, xxviii, p. 25, 1905; P. F. Towbridge and H. S. Grindley: *Ibid.*, xxviii, p. 469, 1906; H. S. Grindley and H. S. Woods: This *Journal* ii, p. 309, 1907.

[2] Grindley and Woods: This *Journal*, ii, p. 309, 1907.

[3] *Pharm. Journ.*, lxxviii, p. 683, 1907.

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Baron Liebig's and Army and Navy) to contain 10 to 12 per cent of combined creatin and creatinin, whereas Bauer and Barschall,[1] and Grindley and Woods[2] found by using Folin's proportion of the reagents 4 per cent and 1 to 6.5 per cents, respectively, of these combined extractives. Such differences in the quantities of creatin and creatinin thus obtained in meat extracts by different analysts certainly demand thorough investigation, if this convenient method of determining creatin and creatinin in such preparations is to be retained. In this connection it should be said, that Hehner gives none of his detailed analytical data and the description of his analytical procedure is so meager that it is impossible to accurately decide the details of his method.

Further, as a result of the work already published by Folin[3] and by Benedict and Meyers[4] in testing the method with pure creatin, Hehner's criticism would at first sight seem to be almost without any foundation. On the other hand, the extent to which this method of determining creatin and creatinin is already being used both in commercial and scientific work demands that the conditions under which it gives accurate results should be fully determined experimentally beyond any reasonable doubt. With this object in view, the work here reported was undertaken.

Folin,[5] in first presenting this method for the quantitative determination of creatinin and creatin in urine, recommended the use of 15 cc. of a 1.2 per cent solution of picric acid and 5 cc. of a 10 per cent solution of sodium hydroxide. Later,[6] in modifying his method he increased the amount of alkali from 5 cc. to 9 cc. on account of the increased quantity of normal hydrochloric acid he found desirable to use in changing the creatin to creatinin. Bauer and Barschall,[7] in their work upon beef extracts, used 15 cc. of the picric acid solution and 5 cc. of the alkali solution. Benedict and Meyers,[8] in using the method with urine and with beef

[1] *Arbeiten aus dem kaiserlichen Gesundheitsamte*, xxiv, p. 552.

[2] *Loc. cit.*

[3] *Zeitschr. f. physiol. Chem.*, xli, p. 223, 1904; *Festschrift f. Olaf Hammarsten*, iii, 1906.

[4] *Amer. Journ. of Physiol.*, xviii, p. 4, 1907.

[5] *Zeitschr. f. physiol. Chem.*, xli, p. 223, 1904.

[6] *Festschrift f. Olaf Hammarsten*, iii, 1906.

[7] *Arbeiten aus dem kaiserlichen Gesundheitsamte*, xxiv, p. 552.

[8] *Loc. cit.*

extracts, took 15 cc. of the saturated picric acid solution and 10 cc. of the alkali solution. In the previous publication[1] upon this subject from this laboratory, 15 cc. of the saturated (1.2 per cent) solution of picric acid and 5 cc. of the 10 per cent sodium hydroxide solution were taken. In the work of this laboratory the details of the method as perfected and thoroughly tested by Folin for the determination of creatinin and creatin in urine were followed as closely as possible. Further, in the previous communication, it was clearly stated that the color of the unknown creatinin picrate solution was compared with 8.0 mm. of the $\frac{N}{2}$ solution of potassium bichromate (24.54 grams per liter) and not with the color obtained by allowing the picric acid and alkali to act upon a standard creatinin solution as Hehner states as the method employed by us. In all our work we have accepted the formulas and equivalents, which were so carefully worked out by Folin.

EXPERIMENTAL.

In testing the method, the influence of varying the quantity of both the picric acid solution and the alkali solution was considered. The differences in the intensity of the color, if there be any within narrow limits, due to the length of time the solutions were allowed to stand were not taken up sufficiently in connection with the present study to warrant consideration in this paper. In all the determinations herein reported, the time allowed for the development of the color was as nearly as possible five minutes. The purity and strength of the picric acid solution were thoroughly tested. The picric acid was found to be quite pure and the strength of the picric acid solution used in this work was 1.2 per cent. A 10 per cent solution of sodium hydroxide was used as the alkali. The volumes of the 1.2 per cent solution of the picric acid used were 15, 30 and 45 cc., and 5, 10 and 15 cc. of the 10 per cent solution of the sodium hydroxide were taken. All the readings of the colorimeter were made by at least two persons each working independently of the other. In making the observations each person recorded three or four of those agreeing most closely. One of the analysts in making the read-

[1] This *Journal*, ii, p. 309, 1906.

ings was not informed as to the nature of the solutions under examination. The average of the readings of the two analysts was taken as the true value for the data reported in the tables here given. In cases where the colorimeter was used continuously for a considerable length of time, the standard bichromate solution was renewed once or twice to avoid any possible error due to evaporation.

Detailed description of the method used. About 10 grams of the commercial meat extract were dissolved in water and the resulting solution was diluted to exactly 500 cc. and thoroughly mixed. In some cases the solutions thus prepared were filtered through dry filters while in other cases the original solutions were not filtered before being used for the determination of the creatinin and the creatin. For the determination of the creatinin, aliquot portions of the sample solution which would give a reading of 7 to 9 mm. in the Duboscq colorimeter were transferred to 500 cc. measuring flasks. To these portions, the measured amounts of the 1.2 per cent picric acid solution and the 10 per cent solution of sodium hydroxide were each added. The mixture was then shaken and allowed to stand exactly five minutes when it was immediately and quickly diluted to the 500 cc. mark with distilled water and thoroughly mixed. Readings upon this solution were made at once in the colorimeter, comparing the depth of color of the solution to be tested with that of a standard half-normal bichromate (24.54 grams per liter) solution set at 8 mm.

For the determination of the creatin measured portions of the sample solution were transferred to small Jena beakers. In case the volumes of the solution taken amounted to more than 10 cc., they were evaporated upon the steam bath to this volume and then treated with 10 cc. of normal hydrochloric acid. The creatin of the solutions was changed to creatinin by the autoclave method of Benedict and Meyers[1] which has proved to be convenient and accurate. To do this, the beakers containing the solutions were placed in an autoclave and heated at a temperature of 117° C.–120° C. for 30 minutes. After these solutions were taken from the autoclave they were diluted to a definite volume

[1] *Amer. Journ. of Physiol.*, xviii, p. 398, 1907.

and measured amounts of the diluted solutions which would give a reading of 7 to 9 mm. in the colorimeter were transferred to 500 cc. measuring flasks. The details of procedure were then continued exactly as directed above for the estimation of the creatinin. The data of the experiments are given below in full.

Experiment 1. For this experiment 9.2899 grams of a well-known brand of commercial beef extract were dissolved in water and the resulting solution was diluted to 500 cc. and thoroughly mixed. The solution was then filtered through dry filters and portions of 25 cc. each were taken for the determination of creatinin by the method described in detail above. The results of the determination, in which varying amounts of the picric acid and the sodium hydroxide solutions were used for the same volume, of the sample solutions are given in the following table:

TABLE 1. CREATININ RESULTS OBTAINED IN EXPERIMENT 1.

Lab. No.	SAMPLE.	METHOD.		Reading of colorimeter.	Weight of creatinin.	Total weight of creatinin.	Per cent of creatinin.
		Picric acid used.	Alkali used.				
		cc.	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*
2278a	Beef extract.....	15	5	9.0	9.000	180.00	1.94
2278a	" "	15	10	8.9	9.101	182.02	1.96
2278a	" "	30	5	8.5	9.529	190.58	2.05
2278a	" "	30	5	8.9	9.101	182.02	1.96
2278a	" "	30	10	8.4	9.643	192.86	2.08
2278a	" "	30	10	8.2	9.878	197.56	2.13
2278a	" "	45	5	9.0	9.000	180.00	1.94
2278a	" "	45	10	8.9	9.101	182.02	1.96
	Average						*2.00*

Experiment 2. For this experiment the same filtered solution of beef extract was taken for the determination of creatin as was used above in Experiment 1 for the determination of creatinin. For the estimation of the creatin in the solution, five portions of 25 cc. each of the original filtered solution were measured out and the creatin which they contained was converted into creatinin in the autoclave as described above. The five solutions representing 125 cc. of the original solution of the extract after removal from the autoclave were united and diluted to 500 cc. Fifty cubic centimeter portions of this diluted solution were taken for the determination of creatinin by the method described in full above. The analytical results obtained, where varying amounts of the picric acid solution and the sodium hydroxide solution were used for the same volume of the sample solution, are given in the following table:

TABLE 2. CREATIN RESULTS OBTAINED IN EXPERIMENT 2.

Lab. No.	SAMPLE.	METHOD.		Reading of colorimeter.	ORIGINAL CREATININ PLUS CREATININ DUE TO CREATIN.			Per cent of original creatinin.	Per cent of creatinin due to creatin.	Per cent of creatin (creatinin due to creatin × 1.16).
		Picric acid used.	Alkali used.		Original weight.	Total weight.	Per cent.			
		cc.	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
2278a	Beef extract.....	15	5	16.1	5.032	201.28	2.17	2.00	0.17	0.20
2278a	" "	15	10	10.5	7.714	308.56	3.32	2.00	1.32	1.53
2278a	" "	15	15	9.6	8.438	337.52	3.63	2.00	1.63	1.89
2278a	" "	30	10	9.0	9.000	360.00	3.88	2.00	1.88	2.18
2278a	" "	30	10	9.1	8.901	356.04	3.83	2.00	1.83	2.12
2278a	" "	30	15	9.0	9.000	360.00	3.88	2.00	1.88	2.18
2278a	" "	30	15	9.2	8.804	352.16	3.79	2.00	1.79	2.08
2278a	" "	45	10	8.9	9.101	364.04	3.92	2.00	1.92	2.23
2278a	" "	45	10	9.0	9.000	360.00	3.88	2.00	1.88	2.18

Experiment 3. For this experiment two solutions were prepared from the same beef extract as that used in Experiments Nos. 1 and 2. In the first, namely, No. 2278b, 11.3131 grams of the extract were dissolved in water and the resulting solution was diluted to 500 cc. and thoroughly mixed. In the second solution, No. 2278c, 10.3424 grams of the extract were dissolved in water and made up to a definite volume of 500 cc. These solutions were not filtered but they were used directly for the work reported in this and the following experiment. Portions of 25 cc. each of these unfiltered solutions were taken for the determination of creatinin.

The results of the tests are given in the following table:

TABLE 3. CREATININ RESULTS OBTAINED IN EXPERIMENT 3.

Lab. No.	SAMPLE.	METHOD.		Reading of colorimeter.	Weight of creatinin.	Total weight of creatinin.	Per cent of creatinin.
		Picric acid used.	Alkali used.				
		cc.	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*
2278b	Beef extract.....	15	5	7.1	11.408	228.16	2.02
2278b	" "	30	5	6.9	11.739	234.78	2.08
2278b	" "	30	10	6.9	11.739	234.78	2.08
2278b	" "	45	5	6.9	11.739	234.78	2.08
2278b	" "	45	10	6.9	11.739	234.78	2.08
	Average.....						*2.07*
2278c	Beef extract.....	15	5	7.8	10.385	207.70	2.01
2278c	" "	15	5	7.8	10.385	207.70	2.01
2278c	" "	15	10	7.8	10.385	207.70	2.01
2278c	" "	15	10	7.9	10.253	205.06	1.98
2278c	" "	21	10	7.8	10.385	207.70	2.01
2278c	" "	21	10	7.8	10.385	207.70	2.01
2278c	" "	21	15	7.8	10.385	207.70	2.01
2278c	" "	21	15	7.8	10.385	207.70	2.01
2278c	" "	30	10	7.8	10.385	207.70	2.01
2278c	" "	30	10	7.8	10.385	207.70	2.01
	Average.....						*2.01*

Experiment 4. For this experiment the same unfiltered solution, No. 2278c, was taken for the determination of creatin as was used above for the determination of creatinin in Experiment 3. For the estimation of the creatin four portions of 25 cc. each of the original unfiltered solution were measured out and the creatin which they contained was changed into creatinin as usual. The four solutions representing 100 cc. of the original solution of the extract after removal from the autoclave were united and diluted to 200 cc. Twenty-five cubic centimeter portions of this diluted solution were taken for the determination of creatinin by the usual method. The results of the tests are given in the following table:

TABLE 4. CREATIN RESULTS OBTAINED IN EXPERIMENT 4.

Lab. No.	SAMPLE.	METHOD.		Reading of colorimeter.	ORIGINAL CREATININ PLUS CREATIN IN DUE TO CREATIN.			Per cent of original creatinin.	Per cent of creatinin due to creatin.	Per cent of creatin (creatinin due to creatin × 1.16).
		Picric acid used.	Alkali used.		Original weight.	Total weight.	Per cent.			
		cc.	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
2278b	Beef extract.....	15	10	8.7	9.310	372.40	3.60	2.01	1.59	1.84
2278b	" " ...	15	10	8.8	9.205	368.20	3.56	2.01	1.55	1.80
2278b	" " ...	21	10	8.7	9.310	372.40	3.60	2.01	1.59	1.84
2278b	" " ...	21	10	8.8	9.205	368.20	3.56	2.01	1.55	1.80
2278b	" " ...	30	10	8.2	9.878	395.12	3.82	2.01	1.81	2.10
2278b	" " ...	30	10	8.2	9.878	395.12	3.82	2.01	1.81	2.10

Experiment 5. For this experiment a cold water extract of 150.5287 grams of lean beef round was prepared by the methods described in a former paper.[1] The resulting extract was made up to a volume of 7500 cc. Five portions of 1000 cc. each of this cold water extract were measured out and evaporated to a volume of about 50 cc. The coagulated proteid resulting was removed by filtration and thoroughly washed with hot water. The filtrates were now evaporated to a volume of 10 cc. and the creatin[2] which they contained was converted into creatinin by use of the autoclave. The five solutions representing five liters of the original cold water extract after removal from the autoclave were united and diluted to 500 cc. Fifteen cubic centimeter portions of this last solution were taken for the determination of creatinin by the usual method. The results of the tests are given in the following table:

TABLE 5. CREATIN RESULTS OBTAINED IN EXPERIMENT 5.

Lab. No.	SAMPLE.	METHOD.		Reading of colorimeter.	Weight of creatinin due to creatin.	Total weight of creatinin (A).	Total weight of creatin. (A × 1.16.)	Per cent of creatin.
		Picric acid used.	Alkali used.					
		cc.	*cc.*	*mm.*	*mgr.*	*mgr.*	*mgr.*	*p. ct.*
2279	Lean beef round.....	15	5	7.3	11.096	554.80	643.57	0.428
2279	" "	15	5	7.7	10.519	525.95	610.10	0.405
2279	" "	15	5	7.4	10.950	547.50	635.10	0.422
2279	" "	15	5	7.5	10.800	540.00	626.40	0.416
2279	" "	15	5†	7.7	10.520	526.00	610.16	0.405
2279	" "	15	10	7.5	10.800	540.00	626.40	0.416
2279	" "	15	10*	7.8	10.380	519.00	602.04	0.400
2279	" "	30	10	7.0	11.570	578.50	671.06	0.446
2279	" "	30	10	7.0	11.570	578.50	671.06	0.446
2279	" "	30	10†	7.2	11.250	562.50	652.50	0.434
2279	" "	30	15	7.0	11.570	578.50	671.06	0.446
2279	" "	30	10	7.0	11.570	578.50	671.06	0.446
2279	" "	45	15	7.0	11.570	578.50	671.06	0.446

* Also added 10 cc. $\frac{N}{1}$ HCl.
† Also added 10 cc. NaCl, 6 per cent.

[1] H. S. Grindley and A. D. Emmett: *Journ. of the Amer. Chem. Soc.*, xxvii, p. 658, 1905.

[2] Former experiments made in this laboratory demonstrated the fact that fresh meats contain only the slightest trace of creatinin if any at all.

Experiment 6. For this experiment five different brands of beef extracts were taken. They were given Laboratory Nos. 2306, 2307, 2308, 2309 and 2310. The following weights of these extracts were respectively taken for the work, 11.7990, 10.1120, 9.5615, 11.2308, and 9.6105 grams. Each of these weighed portions was dissolved in water and the solutions thus obtained were diluted to 500 cc. and thoroughly mixed. The resulting solutions were not filtered but they were used directly for the work reported in this and the following experiment. The portions of these diluted solutions which were taken for the several determinations are given below in the table giving the results of the tests of this experiment.

TABLE 6. CREATININ RESULTS OBTAINED IN EXPERIMENT 6.

Lab. No.	SAMPLE.	Original volume taken.	METHOD. Picric acid used.	METHOD. Alkali used.	Reading of colorimeter.	Weight of creatinin.	Total weight of creatinin.	Per cent of creatinin.
		cc.	*cc.*	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*
2306	Beef extract.....	8.5	15	5	7.5	10.80	635.25	5.38
2306	" "	8.5	15	10	7.4	10.95	644.08	5.47
2306	" "	8.5	30	10	7.2	11.25	661.73	5.61
2306	" "	8.5	30	10	7.3	11.09	652.31	5.53
	Average							*5.45*
2307	Beef extract.....	23.0	15	5	8.0	10.13	220.26	2.18
2307	" "	23.0	15	10	7.8	10.38	225.66	2.23
2307	" "	23.0	30	10	7.6	10.66	231.75	2.29
	Average							*2.23*
2308	Beef extract.....	50.0	15	10	7.8	10.39	103.85	1.09
2308	" "	50.0	21	10	7.8	10.39	103.85	1.09
2308	" "	50.0	21	10	7.8	10.39	103.85	1.09
2308	" "	50.0	30	10	7.8	10.39	103.85	1.09
2308	" "	50.0	30	10	7.8	10.39	103.85	1.09
	Average							*1.09*
2309	Beef extract.....	13.0	15	5	7.3	11.096	426.75	3.80
2309	" "	13.0	15	5	7.3	11.096	426.75	3.80
2309	" "	13.0	15	10	7.2	11.225	432.68	3.85
2309	" "	13.0	15	10	7.0	11.571	445.02	3.96
2309	" "	13.0	30	10	7.0	11.571	445.02	3.96
2309	" "	12.5	30	10	7.3	11.096	443.84	3.95
2309	" "	13.0	30	10	7.1	11.408	438.75	3.91
2309	" "	13.0	30	10	7.1	11.408	438.75	3.91
2309	" "	13.0	30	15	7.1	11.408	438.75	3.91
	Average							*3.89*
2310	Beef extract.....	17.0	15	5	7.0	11.57	340.27	3.54
2310	" "	17.0	15	10	7.0	11.57	340.27	3.54
2310	" "	17.0	30	10	6.9	11.74	344.09	3.58
	Average							*3.55*

Experiment 7. For this experiment the same unfiltered solutions of the five beef extracts used above in Experiment 6 were taken for the determination of creatin. For the estimation of creatin Nos. 2306, 2307, 2309 and 2310, five portions of 25 cc. each were measured from each of the four solutions of the extracts and the creatin which they contained was converted into creatinin as usual. The five solutions representing 125 cc. of the original solutions of each of the extracts after removal from the autoclave were united and diluted to 500 cc. For the estimation of creatin in No. 2308 four portions of 25 cc. each were measured from the original solution of this extract and the creatin contained in the same was converted into creatinin as usual. The four solutions representing 100 cc. of the original solution after removal from the autoclave were united and diluted to 200 cc. The portions of these diluted solutions which were taken for the determination of the creatinin by the usual method are indicated by the following table which gives the detailed analytical results for the three samples of beef extract.

TABLE 7. CREATIN RESULTS OBTAINED IN EXPERIMENT 7.

Lab. No.	SAMPLE.	Diluted sol. taken.	METHOD.		Reading of colorimeter.	ORIGINAL CREATININ PLUS CREATININ DUE TO CREATIN.			Per cent of original creatinin.	Per cent of creatinin due to creatin.	Per cent of creatin (creatinin due to creatin × 1.16).
			Picric acid used.	Alkali used.		Original weight.	Total weight.	Per cent.			
		cc.	*cc.*	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
2306	Beef extract..	50	15	5	7.0	11.571	462.84	3.92	5.45		
2306	" "	50	15	5	7.1	11.408	456.32	3.87	5.45		
2306	" "	30	30	10	7.0	11.571	771.79	6.54	5.45	1.09	1.26
2307	" "	50	15	5	11.0	7.364	294.56	2.91	2.23	0.68	0.79
2307	" "	70	15	10	6.9	11.594	331.23	3.28	2.23	1.05	1.22
2307	" "	70	30	10	6.4	12.656	361.58	3.58	2.23	1.35	1.57
2308	" "	60	30	10	9.0	9.000	150.00	1.57	1.09	0.48	0.57
2308	" "	67	30	10	8.3	9.759	145.70	1.52	1.09	0.43	0.50
2309	" "	37	15	5	9.1	8.901	481.10	4.28	3.89	0.39	0.45
2309	" "	37	15	5	9.0	9.000	486.45	4.33	3.89	0.44	0.51
2309	" "	37	15	5	8.9	9.101	491.91	4.38	3.89	0.49	0.57
2309	" "	37	15	10	8.0	10.125	547.26	4.87	3.89	0.98	1.14
2309	" "	37	15	10	7.9	10.253	554.18	4.93	3.89	1.04	1.21
2309	" "	37	15	10	7.8	10.385	561.31	5.00	3.89	1.11	1.29
2309	" "	37	30	10	7.7	10.519	568.55	5.06	3.89	1.17	1.36
2309	" "	37	30	15	7.9	10.253	554.18	4.93	3.89	1.04	1.21
2310	" "	50	15	5	8.1	10.000	400.00	4.16	3.54	0.62	0.72
2310	" "	50	15	10	5.8	13.966	558.64	5.81	3.54	2.27	2.63
2310	" "	50	30	10	5.6	14.464	578.56	6.02	3.54	2.48	2.88
2310	" "	35	30	10	7.9	10.253	585.45	6.09	3.54	2.55	2.96

Experiment 8. For this experiment three samples of urine from three different persons were taken. These samples were given Laboratory Nos. 2319, 2320 and 2321. The specific gravities of the urines were respectively, 1.033, 1.029, 1.018. In each sample the creatinin was determined by the usual method, using however different quantities of picric acid and varying quantities of alkali. The detailed results of the experiment are given in the table that follows:

TABLE 8. CREATININ RESULTS OBTAINED IN EXPERIMENT 8.

Lab. No.	SAMPLE.	Original volume taken.	METHOD. Picric acid used.	METHOD. Alkali used.	Reading of colorimeter.	Weight of creatinin.	Total weight of creatinin	Per cent of creatinin.
		cc.	*cc.*	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*
2319	Urine.....	4.0	15	5	6.9	11.740	205.45	0.28
2319	"	4.0	15	5	7.0	11.570	202.48	0.28
2319	"	4.0	15	10	6.9	11.740	205.45	0.28
2319	"	4.0	30	10	6.4	12.660	221.55	0.31
2319	"	4.0	30	10	6.5	12.460	218.05	0.30
	Average							*0.29*
2320	Urine.....	4.3	15	5	7.8	10.380	142.41	0.23
2320	"	4.3	30	10	7.4	10.950	150.23	0.25
	Average							*0.24*
2321	Urine.....	6.5	15	5	7.4	10.950	176.78	0.17
2321	"	6.5	15	10	7.3	11.100	179.20	0.17
2321	"	6.5	15	10	7.5	10.800	174.42	0.17
2321	"	6.5	30	10	7.3	11.100	179.20	0.17
2321	"	6.5	30	15	7.3	11.100	179.20	0.17
	Average							*0.17*

Experiment 9. The same samples of urine as were used above in Experiment 8 were taken for the determination of creatin. For the estimation of the creatin in Laboratory Nos. 2319 and 2321 four portions of 10 cc. each were measured from each sample, and the creatin which they contained was changed into creatinin as usual. The four solutions of each sample representing 40 cc. of the original urine after removal from the autoclave were united and diluted to 250 cc. The portions of the diluted solution which were taken for the determination of the creatinin are indicated in the table. For the estimation of creatin in Laboratory No. 2320 three portions of 10 cc. each were taken for changing the creatin into creatinin. The three solutions representing 30 cc. of the original urine after removal from the autoclave were united and diluted to 250 cc. The portions taken for the tests are indicated in the table. The complete analytical data for the creatin determination in the three samples of urine are given in detail in the table below:

TABLE 9. CREATIN RESULTS OBTAINED IN EXPERIMENT 9.

Lab. No.	Sample.	Diluted solution taken.	Method.		Reading of colorimeter.	Original creatinin plus creatinin due to creatin.			Per cent of original creatinin.
			Picric acid used.	Alkali used.		Original weight.	Total weight.	Per cent.	
		cc.	*cc.*	*cc.*	*mm.*	*mgr.*	*mgr.*	*p. ct.*	*p. ct.*
2319	Urine	20.0	15	5	8.4	9.643	210.94	0.29	0.28
2319	"	20.0	15	10	8.2	9.878	216.08	0.30	0.28
2319	"	20.0	15	10	8.2	9.878	216.08	0.30	0.28
2319	"	20.0	30	10	8.1	10.000	218.75	0.30	0.30
2319	"	25.0	30	10	6.4	12.656	221.48	0.31	0.31
2319	"	20.0	30	15	8.3	9.759	213.48	0.30	....
2320	"	31.5	15	5	8.8	9.205	143.69	0.24	0.24
2320	"	31.5	15	10	8.7	9.310	145.33	0.24	0.24
2320	"	31.5	15	10	8.8	9.205	143.69	0.24	0.24
2320	"	25.0	30	10	10.0	8.100	159.33	0.26	0.24
2320	"	31.5	30	10	8.5	9.529	148.75	0.25	0.24
2320	"	31.5	30	10	8.5	9.529	148.75	0.25	0.24
2321	"	34.5	15	10	8.8	9.205	175.08	0.16	0.17
2321	"	34.5	15	10	9.0	9.000	171.18	0.16	0.17
2321	"	34.5	30	10	8.8	9.205	175.08	0.16	0.17
2321	"	34.5	30	10	9.0	9.000	171.18	0.16	0.17
2321	"	25.0	30	10	10.9	7.431	195.06	0.18	0.17

Experiment 10. As a further check on this method, we were fortunate enough to obtain through the kindness of Dr. Folin some pure creatin (Kahlbaum). Crystallized creatin contains one molecule of water of crystallization and in our sample the moisture content was ascertained and duly allowed for in the subsequent data in Table 10. Several portions of the sample, given Laboratory No. 2289, were weighed off and each dissolved in an appropriate quantity of distilled water. The following weights of the dry substance were taken:

(*a*) .1290 grams	(*b*) .2217 grams	(*c*) .1165 grams
(*d*) .2705 "	(*e*) .2217 "	(*f*) .4699 "

All of the samples were dissolved in 100 cc. of distilled water, except in *f*, which was dissolved in 400 cc. From these solutions, five 10 cc. portions of samples, *b*, *d*, *e* and *f*, and nine 10 cc. portions of samples *a* and *c* were transferred to small beakers. To each beaker 10 cc. of normal hydrochloric acid was added, after which the dehydration was carried on at 117–119° C. in the autoclave for one-half hour. Each of the resulting creatinin solutions of the respective samples was transferred to a 500 cc. measuring flask and diluted to the mark. After thoroughly mixing, portions of 50 cc. were taken for the usual colorimetric determinations. Special mention should perhaps be made at this point for subsequent discussion that solutions *a* and *c* contained the equivalent of 90 cc. of normal hydrochloric acid and that the others, *b*, *d*, *e* and *f*, contained 50 cc.

The following table gives the results of the several tests:

TABLE 10. SUMMARY OF RESULTS OBTAINED IN EXPERIMENT 10.

Lab. No.	Weight of dry sample.	Original volume.	Diluted volume taken.	METHOD.		Reading of colorimeter.	Weight of creatinin due to creatin.	Total weight of creatinin due to creatin.	Weight of creatin (Creatinin × 1.16).	Per cent of creatin.
				Picric acid.	Alkali.					
	gms.	*cc.*	*cc.*	*cc.*	*cc.*	*mm.*	*mgr.*	*mgr.*	*mgr.*	*p. ct.*
2289A	0.1290	100	50	15	5	22.6	3.580	39.82	46.19	35.81
2289A	0.1290	100	50	15	5	22.6	3.580	39.82	46.19	35.81
		Average (2)				*22.6*	*3.580*	*39.82*	*46.19*	*35.81*
2289A	0.1290	100	50	15	10	9.1	8.901	98.89	114.72	88.93
2289A	0.1290	100	50	15	10	9.1	8.901	98.89	114.72	88.93
		Average (2)				*9.1*	*8.901*	*98.89*	*114.72*	*88.93*
2289A	0.1290	100	50	30	10	8.4	9.643	107.14	124.29	96.34
2289A	0.1290	100	50	30	10	8.5	9.529	105.88	122.82	95.21
		Average (2)				*8.5*	*9.586*	*106.51*	*123.56*	*95.78*
2289A	0.1290	100	50	30	15	8.5	9.529	105.88	122.82	95.21
2289B	0.2217	100	50	15	10	9.5	8.526	170 52	197.80	89.21
2289B	0.2217	100	50	15	10	9.4	8.617	172.34	199.91	90.17
		Average (2)				*9.5*	*8.572*	*171.43*	*198.86*	*89.69*

Lab. No.	Weight of dry sample.	Original volume.	Diluted volume taken.	METHOD.		Reading of colorimeter.	Weight of creatinin due to creatin.	Total weight of creatinin due to creatin.	Weight of creatin (Creatinin × 1.16).	Per cent of creatin.
				Picric acid.	Alkali.					
2289B	0.2217	100	50	30	10	9.0	9.000	180.00	208.80	94.18
2289B	0.2217	100	50	30	15	9.0	9.000	180.00	208.80	94.18
2289C	0.1165	100	63	15	10	9.0	9.000	89.29	103.58	88.91
2289C	0.1165	100	63	15	10	8.8	9.110	90.37	104.83	89.99
		Average (2)				*8.9*	*9.055*	*89.83*	*104.21*	*89.45*
2289C	0.1165	100	63	30	10	8.5	9.529	94.54	109.66	94.13
2289C	0.1165	100	63	30	10	8.4	9.643	95.67	110.97	95.25
		Average (2)				*8.5*	*9.586*	*95.11*	*110.32*	*94.69*
2289C	0.1165	100	63	30	15	8.5	9.529	94.54	109.66	94.13
2289D	0.2705	100	50	15	5	9.0	9.000	180.00	208.80	76.89
2289D	0.2705	100	50	15	10	7.7	10.519	210.38	244.04	90.21
2289D	0.2705	100	50	15	10	7.6	10.658	213.16	247.27	91.04
		Average (2)				*7.7*	*10.589*	*211.77*	*245.65*	*90.63*
2289D	0.2705	100	50	30	10	7.2	11.250	225.10	261.11	96.49
2289D	0.2705	100	50	30	10	7.3	11.096	221.92	257.43	95.17
2289D	0.2705	100	50	30	10	7.3	11.096	221.92	257.43	95.17
2289D	0.2705	100	50	30	10	7.4	10.946	218.92	253.95	93.88
		Average (4)				*7.3*	*11.097*	*221.97*	*257.48*	*95.18*
2289D	0.2705	100	50	30	15	7.4	10.946	218.92	253.95	93.88
2289E	0.2217	100	50	15	5	11.1	7.300	145.94	169.29	76.36
2289E	0.2217	100	50	15	10	9.0	9.000	180.00	208.80	94.18
2289E	0.2217	100	50	15	10	8.9	9.101	182.02	211.14	95.28
		Average (2)				*9.0*	*9.051*	*181.01*	*209.97*	*94.71*
2289E	0.2217	100	50	30	10	8.9	9.101	182.02	211.14	95.24
2289E	0.2217	100	50	30	10	8.9	9.101	182.02	211.14	95.24
2289E	0.2217	100	50	30	10	8.9	9.101	182.02	211.14	95.24
		Average (3)				*8.9*	*9.101*	*182.02*	*211.14*	*95.24*
2289F	0.4699	200	50	15	5	10.2	7.940	317.64	368.46	78.47
2289F	0.4699	200	50	15	5	10.4	7.788	311.52	361.36	76.88
		Average (2)				*10.3*	*7.864*	*314.58*	*364.91*	*77.68*
2289F	0.4699	200	50	15	10	8.4	9.643	385.72	447.44	95.22
2289F	0.4699	200	50	15	10	8.3	9.759	390.36	452.82	96.38
		Average (2)				*8.4*	*9.701*	*388.04*	*450.13*	*95.80*
2289F	0.4699	200	50	30	10	8.0	10.125	405.00	469.80	99.98
2289F	0.4699	200	50	30	10	8.0	10.125	405.00	469.80	99.98
		Average (2)				*8.0*	*10.125*	*405.00*	*469.80*	*99.98*
2289F	0.4699	200	50	30	15	8.0	10.125	405.00	469.80	99.98

Experiment 11. For this experiment several tests were made upon solutions of creatinin (Merck's). This product was found to be only about 85 per cent pure, there being also present approximately 10 per cent of creatin and 2.07 per cent of moisture. As a result, the material in question could not be used to serve the double purpose intended, namely, the checking of the method and the standardizing of the bichromate solution.

However, to ascertain the variations, if any, in the percentage of creatinin in the sample when different proportions of the picric acid and alkali were used three portions were weighed out. Sample *a* weighed 0.1286 grams (dry); *b*, 0.1127 grams (dry), and *c*, 0.2055 grams (dry). The first two samples were dissolved in 500 cc. of distilled water and after thoroughly mixing, 50 cc. were taken for the usual procedure. Sample *c* was dissolved in 250 cc. of water and 100 cc. of this solution were taken and diluted to 500 cc. after which 75 cc. were used for the regular determination.

The detailed data resulting are given in the following table:

TABLE 11. SUMMARY OF RESULTS OBTAINED IN EXPERIMENT 11.

Lab. No.	Weight of sample.	Original volume.	Original or diluted volume taken.	METHOD.		Reading of colorimeter.	Weight of Creatinin.	Total weight of creatinin.
				Picric acid	Alkali.			
	gms.	*cc.*	*cc.*	*cc.*	*cc.*	*mm.*	*mgr.*	*mgr.*
2353a	.1286	500	50	15	5	8.7	9.310	93.10
2353a	.1286	500	50	15	5	8.8	9.205	92.05
2353a	.1286	500	50	15	10	7.6	10.658	106.58
2353a	.1286	500	50	15	10	7.6	10.658	106.58
2353a	.1286	500	50	30	10	7.4	10.946	109.46
2353a	.1286	500	50	30	10	7.5	10.800	108.00
2353a	.1286	500	50	30	15	7.4	10.946	109.46
2353a	.1286	500	50	30	15	7.5	10.800	108.00
2353b	.1127	500	50	15	5	10.2	7.941	79.41
2353b	.1127	500	50	15	10	8.8	9.205	92.05
2353b	.1127	500	50	15	10	8.8	9.205	92.05
2353b	.1127	500	50	30	10	8.6	9.415	94.15
2353b	.1127	500	50	30	10	8.7	9.310	93.10
2353b	.1127	500	50	30	15	8.8	9.205	92.05
2353c	.2055	250	75	15	10	9.0	9.000	150.03
2353c	.2055	250	75	15	10	9.0	9.000	150.03
2353c	.2055	250	75	30	10	8.0	10.125	168.78
2353c	.2055	250	75	30	10	8.0	10.125	168.78
2353c	.2055	250	75	30	15	8.0	10.125	168.78

DISCUSSION OF RESULTS.

It is quite apparent from the data reported in the preceding pages that the Folin method of estimating creatinin and creatin, when properly modified, is as applicable to meat and meat extract as it is to urine. Further, it is evident that if the details of the method are properly followed, reliable and concordant results can be obtained.

ORIGINAL CREATININ. (*a*) *Influence of alkali.* By comparing the data in Tables 1, 3, 6 and 8 which relate to the percentage of creatinin in meat extracts and urine, it is seen that in so far as the initial quantity of creatinin is concerned, the influence of increasing the amount of alkali is practically nil. If 15, 21 or 30 cc. of picric acid of 1.2 per cent are used with either 5, 10 or 15 cc. of sodium hydroxide of 10 per cent strength the readings of the colorimeter for the *same* volume of the solutions under examination, are in general slightly higher for the 5 cc. quantities than for the 10 cc. portions. Stating these facts in terms of percentages of creatinin, the 10 and 15 cc. quantities of sodium hydroxide, which are approximately 8 and 13 cc., respectively, in excess of the necessary amount to cause neutralization and solution of the precipitate, gave but very slightly higher percentages than did the 5 cc. quantities. These actual differences in the case of meat extracts were 0.02 to 0.17 per cent, being on the average 0.05 per cent. In the case of the creatinin (Merck's), which as previously stated contained 10 per cent of creatin, the data in Table 11, show the variations in amount of alkali to produce an appreciable difference. The weights of original creatinin resulting from the use of 10 or 15 cc. of alkali are the same, but for those resulting from the use of 5 cc., they are distinctly lower, being in the former cases on an average of 106.58 mgms., and in the latter 92.58 mgms., a difference of 14.0 mgms. No positive explanation can be given for this large variation. It is easily seen that the differences in some cases in the readings due to the amounts of alkali might be from 2 to 4 mm. as was the case with the beef extracts and urine, and this would mean with such a strong solution, a difference of 2 to 4 per cent.

From the above discussion, the data show that, contrary to Hehner's statement, an excess of alkali, using 5, 10 and 15 cc. of

a 10 per cent solution, does not diminish the depth of the color produced with creatinin picrate but rather increases it. It would seem, therefore, that it would be best to use 10 cc. of a 10 per cent solution of alkali in all instances, it having been shown that this amount of alkali has no detrimental effect whatever.

(*b*) *Influence of picric acid.* In studying the data in Tables 1, 3 and 6 on beef extract, 8 on urine, and 11 on pure creatinin, it will be seen that the variations in the percentages of original creatinin are extremely slight when the quantity of picric acid is increased from 15 to 21, 30 or 45 cc. in the cases where 10 cc. of the alkali are used. In fact, it can be stated, as far as the creatinin sample and also the meat extract samples are concerned, that the 15 cc. test of picric acid (1.2 per cent) gave as high a percentage of creatinin as did the 21 cc. test (which is the equivalent of Hehner's 25 cc. portion, 1.01 per cent), or even the 30 cc. test. This fact is most clearly brought out in Tables 3 and 6 where the analysis of six different samples of meat extract are reported. Sample 2306 has a variation, taking the average of the readings of the 30 cc. tests, of 0.10 per cent on a total of 5.47 per cent of original creatinin. Sample 2307 shows an increase of 0.06 per cent for the 30 cc. of the picric acid against that of 15 cc. on a total of 2.23 per cent. Similarly, the data for sample 2310 gave a gain of 0.04 per cent for the 30 cc. of picric acid when compared with the 15 cc. on a percentage of creatinin of 3.55. These slight differences are no greater than the errors which might be due to the matching of the color, to the carrying out of the technique of the method or to the sampling of the products.

In the case of the urine, Table 8, the variations in the readings for sample 2319 are greater than in the cases just considered but those for sample 2321 show the same variations as the meat extracts. When calculated to the percentage of creatinin these differences in the urine are insignificant. Further, when the analysis of the three samples of the pure creatinin are considered, it is seen that the readings of the two tests, 15 and 30 cc. of picric acid are almost identical. These small differences are no greater than would be expected. However, if these values are calculated to percentages, the variations are about 1.0 per cent. The cause of this difference is not to be found as Hehner

states in the greater amount of creatinin in the solution which is being treated with picric acid, because in this, and the previous work of this laboratory, whether the sample under examination was meat extract, urine, or creatinin itself, such an aliquot portion of the *original* solution was taken that when it was treated in the usual manner with picric acid and alkali, and diluted, the resulting color gave a reading of 7 to 9 mm. on the scale of the colorimeter when compared with the standard bichromate set at 8 mm. In other words, all the solutions tested at the time of the reading contained approximately the same quantity of original or converted creatinin. The real cause of the influence of the slight differences in the readings upon the variations calculated in percentage, naturally lies in the differences in the weights of the substances taken for the samples, since the proportion of the dilutions were approximately the same. The weights of the samples were always about 150 grams for meats, 10 grams for meat extracts and 0.12 grams for the creatinin, and, consequently, it is plainly seen that any variation in the readings would be very much less apparent in the percentage composition in the case of the meats than in the meat extract or the impure creatinin.

From this discussion upon the influence of varying the quantity of picric acid in cases where 10 cc. of a 10 per cent solution of alkali were used, it seems safe to state that it makes no difference, when determining the original creatinin, whether 15, 21 or 30 cc. of the 1.2 per cent solution of picric acid are used. The final percentages are practically identical.

Creatinin due to Creatin. (*a*) *Influence of alkali.* In the former paper, it was stated that the creatin in the samples was changed to creatinin by using 25 cc. of $\frac{N}{10}$ hydrochloric acid and that the resulting solutions after proper dilution were treated with 15 cc. of picric acid (1.2 per cent) and 5 cc. of alkali (10 per cent). The data herein reported were ascertained by using 10 cc. of normal hydrochloric acid for the dehydration or four times the amount previously taken. Following this change in the amount of acid, the quantity of alkali was also increased. These modifications were adopted on account of the recommendations of Folin in his second paper,[1] that in the determining of creatin in

[1] *Festschrift f. Olaf Hammarsten*, iii, 1906.

abnormal urine 10 cc. of normal hydrochloric acid, 15 cc. of picric acid (1.2 per cent) and 9 cc. of sodium hydroxide (10 per cent) should be taken.

In studying the influence of different proportions of alkali when varying amounts of dehydrated creatin and original creatinin were present, two series of tests were carried out. In the first, 5 and 10 cc. of the alkali were used against 15 cc. of the picric acid, and in the second, 10 and 15 cc. of the alkali were employed with 30 and sometimes 45 cc. of the picric acid. It was ascertained in the case of meat extracts that approximately 1 to 2 cc. of the 10 per cent alkali were sufficient to dissolve the precipitate and to produce a red coloration.

The data in Tables 2, 4 and 7, relating to meat extracts, show the effect of using 5 and 10 cc. of the alkali with 15 cc. of the picric acid. It is very apparent that the 5 cc. portion is entirely too small. This fact is perhaps shown most markedly in sample 2306, where the total creatinin is 3.90 per cent while that for the original creatinin is 5.45 per cent. In other words, the 5 cc. of alkali produced a color which, when compared with the standard, represented a value of 29.4 per cent less than the amount obtained before dehydration. A comparison of the 5 and 10 cc. tests is best brought out in the case of samples 2278a, Table 2; and 2309 and 2310, Table 7. The differences in the readings with the two quantities of alkali vary from 1.1 mm. in 2309 to 5.6 mm. in 2278a. These data when calculated to the percentage of creatinin as creatin, show a range of 0.7 to 1.33, respectively, in favor of the 10 cc. portion of alkali.

Very little can be said regarding the influence of the quantity of alkali in the case of the meats and urines since the data are too few for consideration. The slight differences in those instances that are reported are inappreciable when calculated to their final percentages. However, in connection with the meat extract the facts show very plainly that the 10 cc. of alkali when used with 15 cc. of picric acid gives higher results than the 5 cc. portion.

In the second trial where 10 and 15 cc. of alkali were used with 30 or 45 cc. of picric acid, the resulting data for the beef extracts and meat are fairly constant. The few variations amount in the maximum to only 0.2 mm. This would indicate that the slight differences were not due to the excess of alkali, but rather

to errors in the technique. However, it should perhaps be stated that these differences are in the main in favor of the lower readings for the 10 cc. test, but when the data in Table 10, relating to pure creatin, are also taken into account the evidence seems to be such that it can be stated that there are no differences resulting in using either 10 or 15 cc., of alkali with 30 cc. of the picric acid, beyond the experimental errors.

From the above consideration, the data show that the quantity of alkali does influence the depth of color, that a small quantity does not yield as high a percentage as a large one; that a large excess does not give low results, and that the accepted 10 cc. portion, yields better results than the 5 cc. and similar results to the 15 cc. portion.

(*b*) *Influence of picric acid.* In the preceding pages, the effect of using varying amounts of alkali and picric acid has been considered where preformed creatinin was to be determined, and in the above paragraphs, the influence of different quantities of alkali has been discussed in the case where both the preformed and dehydrated creatinin were present. It is the purpose of this section to digest the data herein reported which refer directly to the effect of using larger quantities of picric acid than has been customary in determining creatin as creatinin.

Hehner noticed in his work that an increase of the picric acid from 15 cc. to 25 cc. (1.01 per cent) caused a marked difference in the amount of creatinin found to be present in meat extracts. Instead of obtaining with 25 cc. of picric acid (1.01 per cent) 6 to 7 per cent of creatinin, which he got with the equivalent of 15 cc. of 1.2 per cent acid, he reported 10 to 12 per cent. He found, further, that a larger amount of picric acid had no increased effect.

In our work, the amounts of picric acid used were 15, 21, 30 and 45 cc., and since it was found that 10 cc. of the sodium hydroxide worked satisfactorily, this quantity of alkali was taken throughout for the comparison. The data in Tables 2, 4, and 7 on meat extracts, Table 5 on meat, and Table 10 on pure creatin, show that in the case of 30 cc. of the picric acid the general tendency is to produce a lower reading and hence a higher percentage of creatinin as creatin. These differences in the readings for the meat extracts are practically nothing in sample 2309

and 1.4, 0.6, 0.5 and 0.2 mm. in samples 2278a, 2278b, 2307 and 2310, respectively; for the meat they are 0.5 mm., and for the pure creatin 0.1 to 0.6 mm. Concerning the data for the urine, Table 9, the differences due to the increased amount of picric acid are very slight, being in samples 2319 and 2321 almost nothing and in sample 2320, 0.3 mm.

In general then, it may be stated for the meat extracts, meat and pure creatin that the additional quantity of picric acid may have no effect in some cases and in others it may cause a decrease in the readings of 0.2 to 1.4 mm. From the data in the following Table 12, which gives a summary of these facts, the differences

TABLE 12. SUMMARY OF RESULTS ON INFLUENCE OF PICRIC ACID.

(*Creatin.*)

Lab. No.	SAMPLE.	READINGS OF COLORIMETER.			PERCENTAGE OF CREATIN.		
		Picric acid 15 cc.	Picric acid 30 cc.	Difference.	Picric acid 15 cc.	Picric acid 30 cc.	Difference.
		mm.	*mm.*	*mm.*	*p. ct.*	*p. ct.*	*p. ct.*
2278a	Beef extract.....	10.5	9.1	1.4	1.53	2.15	0.62
2278c	" "	8.8	8.2	0.6	1.82	2.10	0.28
2307	" "	6.9	6.4	0.5	1.22	1.57	0.35
2309	" "	7.9	7.8	0.1	1.29	1.36	0.07
2310	" "	5.8	5.6	0.2	2.88	2.96	0.08
2279	Meat.....	7.5	7.0	0.5	0.42	0.45	0.03
2319	Urine.....	8.2	8.1	0.1	0.30	0.30	0.00
2320	"	8.8	8.5	0.3	0.24	0.25	0.01
2321	"	8.9	8.9	0.0	0.16	0.16	0.00
2289a	Pure creatin.....	9.1	8.5	0.6	88.93	95.78	6.85
2289b	" "	9.5	9.0	0.5	89.69	94.18	4.49
2289c	" "	8.9	8.5	0.4	89.45	94.69	5.24
2289d	" "	7.7	7.3	0.4	90.63	95.18	4.55
2289e	" "	9.0	8.9	0.1	94.71	95.24	0.53
2289f	" "	8.4	8.0	0.4	95.80	99.98	4.18

in the percentage of creatin in the meat extracts are seen to vary from 0.07 to 0.62, the total percentage of creatin being 1.4 to 3.0. The meat shows a corresponding difference, a gain of 0.03 per cent on a total of 0.446 per cent. In the case of the urine the variations are practically nothing, and in that of the pure

creatin, they are on the average 4.4 per cent greater for the 30 cc. portion of acid.

A second point should be considered, as to the influence of using still more of the picric acid. The data for the sample of beef extract 2278a, Table 2, and that for the meat 2279, Table 5, show that an additional 15 cc. of acid, or 45 cc. in all, does not cause any further change than that brought about by the 30 cc. test.

The above two facts agree in general with Hehner's conclusions that 25 cc. (1.01 per cent) of picric acid should be used and that an additional amount produces no different effect. However, mention should be made that in our case where 21 cc. of the 1.2 per cent picric acid, which is the equivalent of 25 cc. of a 1.01 per cent solution was used, no decided change was produced. This fact is shown in the data in Table 4. Further, while the additional amount of picric acid seems in general to cause a deeper color, the authors wish to emphasize the fact that this difference is by no means as great as Hehner states it to be in his paper. After calculating the data, obtained by using 30 cc. of picric acid and 10 cc. of alkali, it will be seen that the combined percentages of creatinin and creatin in the six different samples of beef extract reported in Tables 2, 4, and 7 amount to 4.15, 4.11, 6.71, 3.80, 1.63, 5.24 and 6.46. These samples correspond, respectively, to those for Laboratory Nos. 2278a, 2278c, 2306, 2307, 2308, 2309 and 2310. When these percentages are compared with those reported in the previous paper which ranged from 1.38 to 6.56 per cent, it can be stated that the data do not differ materially in the two cases, and in as much as the samples used for this work are both representative of those reported formerly and also of Hehner's the evidence seems to indicate that Hehner's results which varied from 10 to 12 per cent for the combined creatinin and creatin were entirely too high.

From the above consideration of the data, it is evident that in the majority of cases the method gives higher results for the converted creatin in meat extracts, meat and pure creatin when the quantity of picric acid (1.2 per cent) is increased from 15 to 30 cc., although several instances are reported where the smaller amount of acid served equally well. No definite explanation can be given at the present time for this apparent anomaly. The

tendency seems to indicate that it is more difficult for the 15 cc. of picric acid to overcome the resistance of the resulting converted creatinin than that of the preformed creatinin which shows that the former must exist in a different condition than the latter. The amount of hydrochloric acid used in dehydrating the creatin does not seem to influence the results as is shown in Experiment 10 which relates to the pure creatin. Here, some solutions had 90 cc. of hydrochloric acid and others 50 cc., yet the general effect of the picric acid was the same in each case. Jaffe,[1] states that by using the zinc chloride method his maximum yield was 94.83 per cent and adds that this seems to show that the creatinin resulting from converted creatin is broken down to some extent by the strong acid. In general the results reported in this paper confirm Jaffe's conclusion. Benedict and Meyers[2] in using Folin method with creatin obtained from 96 to 98.9 per cent, while the data here reported show a variation in six determinations of from 94.2 per cent to 95.8 per cent, and in one test the result was 99.98 per cent. These facts, however, do not necessarily reflect upon the Folin colorimetric method as applied to urine, meat and meat extract. Since normal urine contains no creatin, meats only 0.44 per cent, and meat extracts from 1 to 6 per cent, it will be seen that a yield of 95 per cent is sufficiently accurate for all practical purposes. Further, in as much as the modified method seems to give uniform results throughout, the data should be comparable in all cases and be of extreme value in giving important information as to the quantity of these extractives in meat products.

OUTLINE OF METHOD AS NOW USED IN THIS LABORATORY.

The following brief outline of the method as now used is given: For the preformed creatinin, transfer aliquot portions of the sample solution to 500 cc. measuring flasks, add 15 cc. of a 1.2 per cent picric acid solution, mix, add 10 cc. of a 10 per cent sodium hydroxide solution, shake thoroughly, and allow the mixture to stand 5 minutes and then dilute to the mark at once and after mixing, compare the depth of the color of the solutions with that

[1] *Zeitschr. f. physiol. Chem.*, xlviii, p. 436, 1906.
[2] *Amer. Journ. of Physiol.*, xviii, p. 4 1907.

of a half-normal bichromate solution set at 8 mm. According to Folin, the correct reading in millimeters of the colorimeter divided into 81 gives the number of milligrams of creatinin contained in the portion of the solution taken for the treatment with picric acid and sodium hydroxide. In other words, 10 milligrams of pure creatinin after the addition of the picric acid and the alkali and dilution to 500 cc., gives a reading of 8.1 mm. when compared with 8 mm. of $\frac{N}{2}$ potassium bichromate solution (24.54 grams per liter). For the combined creatinin, transfer aliquot portions of the sample solution to beakers, if the quantity is more than 10 cc., or to 100 cc. measuring flasks, if the quantity is 10 cc. or less. In the former instance, evaporate the solution on the water-bath to 10 cc. In either case make the volume of the liquid up 10 cc., if necessary, and add 10 cc. of normal hydrochloric acid. Rotate the vessels to mix the liquids. Transfer the acid solutions to an autoclave and heat them at a temperature of 117 to 119° C. for 30 minutes. After removal, cool and dilute to the mark. If beakers were used, transfer the contents to 100 cc. measuring flasks and dilute. To aliquot portions of the converted creatinin solution add, in 500 cc. flasks, 30 cc. of 1.2 per cent picric acid, shake and then add 10 cc. of the 10 per cent sodium hydroxide. Mix thoroughly and after standing exactly 5 minutes dilute, and read the depth of color of the solution. In order to convert milligrams of creatinin into creatin multiply by the factor 1.16.

It was found that by using a black cloth, to shut out the surrounding light from the eyepiece of the instrument, the colors appeared more distinctly, and that the comparison could be made more accurately and rapidly and with less strain on the eye.

CONCLUSIONS.

From this study upon meat extracts and meat, the following conclusions can be made in regard to the applicability of the Folin method for determining creatinin and creatin:

(*a*) That an increase in the quantity of picric acid, according to Hehner's suggestion causes no difference in the so-called original creatinin determinations; but it generally does produce an appreciable difference when the converted creatin is also

present, and, further, that the quantity of picric acid (1.2 per cent) recommended for use in meat extract, meat and urine should be left at 15 cc. for the original creatinin determinations and be increased to 30 cc. for the dehydrated creatinin.

(*b*) That in the determination of the original creatinin, the use of a small or large amount of 10 per cent alkali makes almost no difference, the 5 cc. quantity giving slightly lower results than the 10 and 15 cc. quantities; that, for the converted creatin, the previously accepted quantity of alkali, 10 cc., gives better results than 5 cc. and equally as good results as the large excess, 15 cc.; and further, that these facts are contrary to those found by Hehner who states that a quite small amount of alkali gives better results than a large quantity which he maintains diminishes the depth of color.

(*c*) That the data reported are representative of the percentages of creatinin and creatin in meats and meat extracts, being practically the same for the combined extractives as those previously published, 0.45 per cent for the former and 1.4 to 6.5 per cent for the latter, whereas Hehner found the total percentage of creatinin and creatin in meat extracts to be 10 to 12 per cent.

(*d*) That the Folin method when properly modified is as applicable to meat extracts and meats as it is to urine, and that it gives reliable and concordant results in the hands of different analysts of this laboratory.

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ERRATA, VOLUME III.

Page 85, line 26, for *exclusive* read *extensive*.

Page 90, line 7, for *chloride* read *hydroxide*.

Page 180, Nos. (3), (4), (5) and (6) of table of acetone determinations should read, "20 cc. acetone solution + 0 cc. H_2O, etc."

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